

AMENDMENTS TO THE SPECIFICATION

Please amend the specification as shown:

Please delete the paragraph on page 13, line 17 to page 14, line 13 and replace it with the following paragraph:

Figure 5 depicts population sequencing and LigAmp analysis of HIV-1 in plasma samples. (a) Plasma HIV-1 from subjects 242, 842, and 109 were sequenced with the ViroSeq system (population sequencing). Those sequences (SEQ ID NOS 33-36, respectively, in order of appearance) were aligned with sequences of the HIV-1 upstream WT and downstream common oligonucleotides (arrows) using MegAlign (DNASar, Madison, WI). A consensus sequence is shown at the top (SEQ ID NO: 32). Nucleotides at the third position of codon 103 (bracket) are boxed. An A→G substitution at the first base of codon 103 in the upstream wild-type and mutant oligonucleotides (underlined) enhances specificity of the ligation reaction. Dots indicate nucleotides that match the consensus sequence. Nucleotide mixtures are indicated using IUB codes. (b-c, left) Plasma HIV-1 was analyzed using the ViroSeq system. Electropherograms show sequences near codon 103 in HIV-1 reverse transcriptase. Arrowheads indicate the orientation of ViroSeq sequencing primers. The sequence of the HXB2 reference strain ("Ref") is shown above the sequence of each sample. Amino acids encoded by the reference sequence (above) and the sample sequence (below) are shown at the top of each panel. The nucleotide at the third position of codon 103 is boxed. A mixture of nucleotides (A and G = R) is present at this position in the sequence from subject 109 (c); the lower case designation (r) indicates that the nucleotide sequence was manually edited. (b-c, right) Q-PCR amplification curves from subjects 242 and 109, respectively. Curves were generated using either the mutant (Mut, red) or wild-type (WT, blue) upstream oligonucleotide. (d) Plasma from subject 242 (mostly HIV-1 with the K103N mutation) was serially diluted with plasma from subject 109 (mostly wild-type HIV-1). The percentage of plasma from subject 242 in the samples was 100%, 10%, 1%, 0.1%, 0.01% and 0%, all at 50,000 copies/ml HIV-1 RNA. DNA was amplified from the plasma mixtures using the ViroSeq system. LigAmp was performed using the upstream mutant oligonucleotide for detection of K103N. Ct

values were plotted against the dilution of sample 242 (red dots and line). The Ct of the sample of plasma from subject 109 only (blue dot, arrow) was similar to the Ct obtained with a 1:1,000 dilution of plasma from subject 242.

Please delete the paragraph on page 17, lines 15-24 and replace it with the following paragraph:

Figure 16 depicts the results of LigAmp in the detection of methylated DNA. DNA methylation has been used as a biomarker to distinguished tumors from normal tissues. The most widely used assay for detection of methylation levels is methylation-specific PCR (MS-PCR). In MS-PCR, unmethylated cytosines are first converted into thymine by sodium bisulfate treatment, while methylated cytosines in CpG islands are refractory to the treatment, thereby converting methylation information into sequence difference. The DNA is then amplified using primers designed specifically for methylated or unmethylated DNA. LigAmp was designed to detect point mutations. Single base differences between methylated and unmethylated DNA are created after bisulfate modification. Figure 16A discloses SEQ ID NOS 37-38, 38, 39, 40, 40 and 39, respectively, in order of appearance.

Please delete the paragraphs on page 19, line 25 to page 20, line 14 and replace them with the following paragraphs:

Figure 22 depicts detection of the GAT KRAS2 mutation in serum from biliary tract cancer patients. A. The relative amount of the mutant to wild-type KRAS2 DNA in serum detected by LigAmp. Error bars are 1 standard deviation from 3 independent LigAmp assays.. B. DNA sequence (SEQ ID NO: 41) of the cloned BstN1 refractory PCR product from sample 166, confirming the existence of a low level GAT mutation.

Figure 23. Depicts HIV-1 sequences (SEQ ID NOS 43-53 and 55-67, respectively, in order of appearance) from Ugandan women and infants. Paired plasmids with and without the K103N mutation were used as reference reagents to optimize the LigAmp assay for detection of K103N in subtype A and D HIV-1. The sequences of five subtype A plasmids (Panel A, 667, 842, 847, 687 and 703) and three subtype D plasmids (Panel B, 868, 638, 607) are shown (control plasmids with K103N).

Nucleotides at the third position of codon 103 (bracket) are boxed. The sequences of the binding regions of the ligation oligonucleotides used in the first step of the LigAmp assay are shown at the top of each alignment (Oligos). An A→G substitution at the first base of codon 103 in the upstream oligonucleotide (underlined) enhances specificity of the ligation reaction. The lower section of each alignment shows sequences obtained using the ViroSeq system (population sequences) from Ugandan women (M) and infants (I) 6-8 weeks after single dose NVP. A consensus sequence (SEQ ID NOS 42 and 54, respectively, in order of appearance) is shown above each alignment. Dots indicate nucleotides that match the consensus sequence. Nucleotide mixtures are indicated using IUB codes: M=A+C, R=A+G, Y=C+T, H=A+C+T, W=A+T.

Please delete the paragraph on page 67, lines 21-27 and replace it with the following paragraph:

KRAS2 sequencing

We PCR amplified the *KRAS2* locus using M13-tailed primers (5'-GTAAAACGACGGCCAGG-GAGAGAGGCCTGCTGAAAA-3' (SEQ ID NO: 1) and 5'-CAGGAAACAGCTATGACT-TGGATCATATTCGTCCACA-3' (SEQ ID NO: 2), M13 tails underlined). M13 primers were used for sequencing, the BigDye Terminator 3.1 Cycle Sequencing Kit and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Please delete the paragraph on page 76, lines 13-20 and replace it with the following paragraph:

KRAS2 sequencing

KRAS2 sequencing was performed on pancreatic cancer DNA by PCR amplifying the *KRAS2* locus (including codon 12) using the upstream 5'-GTAAAACGACGGCCAGG-GAGAGAGGCCTGCTGAAAA-3' (SEQ ID NO: 1) and downstream 5'-CAGGAAACAGCTATGACT-TGGATCATATTCGTCCACA-3' (SEQ ID NO: 2) M13 tailed (underlined regions) primers. Sequencing was then performed using M13 forward or reverse primers, BigDye 3.1 and a 3100 capillary sequencer (ABI, Applied Biosystems (Foster City, CA).

Please delete the Table 1 header on page 76, line 28 and replace it with the following header:

Table 1. Oligonucleotides and probes (SEQ ID NOS 3-12, respectively, in order of appearance)

Please delete the Table 2 header on page 78, line 1 and replace it with the following header:

Table 2. Oligonucleotides and probes (SEQ ID NOS 3-4, 6-7, 13-17 and 9-12, respectively, in order of appearance)

Please delete the paragraph on page 79, line 9 to page 80, line 2 and replace it with the following paragraph:

LigAmp Assay

A region of *KRAS2* including *KRAS2* codon 12 (hot spot) was first PCR amplified using 5'-GGAGAGAGGCCTGCTGAAAA-3' (SEQ ID NO: 18) and 5'-AATGATTCTGAATTAGCTGTATCGTCA-3' (SEQ ID NO: 19) primers. For CGT *KRAS2* mutation, the mutant *KRAS2* DNA was amplified from a plasmid containing the mutant *KRAS2* sequence. GTT, GAT and wild-type *KRAS2* DNA were amplified from SW480, LS513 and Hela cell genomic DNA, respectively. To construct standard quantitative curves for mutant DNA, amplified mutant *KRAS2* from the cell lines was serially diluted into wild-type DNA. Wild-type *KRAS2* DNA was also serially diluted to quantify amounts of wild-type DNA. Concentrations of standard mutant and wild-type *KRAS2* DNA were determined using NanoDrop (NanoDrop Technologies, Wilmington, DE). Juice amplified *KRAS2* DNA were diluted at 1/100 prior to LigAmp analysis. Mutant DNA mixtures and diluted DNA were incubated with ligation oligonucleotides and 4 U *Pfu* DNA ligase in 1x *Pfu* Ligase Buffer (Stratagene, La Jolla, CA). Ligation reactions were first denatured at 95 °C for 3 min, and then incubated for 90 two-step cycles of 95 °C for 30 seconds alternating with 65 °C for 4 min. To simultaneously determine mutant and wild-type *KRAS2*, both wild-type and mutant upstream oligonucleotides were included in the reaction. The concentrations for mutant upstream

and common downstream oligonucleotides were 1 pmol and 0.5 pmol, respectively, while the concentration for the wild-type upstream oligonucleotide was reduced to 1 fmol. This maintained the full range of mutant DNA detection.

Please delete the paragraphs on page 80, line 10 to page 81, line 3 and replace them with the following paragraphs:

Restriction digestion analysis of KRAS2 PCR products

To eliminate wild-type and enrich for mutant *KRAS2*, *KRAS2* DNA was first amplified using a forward mutant primer that produces a BstN1 restriction enzyme recognition site when the wild-type allele is amplified [Mitchell, 1995 #41] (F: 5'-AATATAAACTTGTGGTAGTTGGACCT-3' (SEQ ID NO: 20), R: 5'-TCAAAGACAAGGCGATATGC T-3' (SEQ ID NO: 21), underlined cytosine introduced to create the BstN1 site). A 30-cycle PCR using AmpliTaq Gold[®] DNA polymerase (ABI) was performed, yielding a 1031 bp PCR product. A second BstN1 site that cuts both the wild-type and mutant *KRAS2* is 136 bases upstream of the reverse primer (mutant: 136 and 906 bases; wild-type: 18, 136 and 888 bases).

Following purification using Qiagen PCR purification Kit, the PCR product (5 µl) was digested with BstN1 (20 units, NEBL, New England Biolabs, Beverly, USA) at 65 °C for 2 hours, and analyzed by 2% agarose gel. To confirm co-existence of a minor mutation in the presence of a dominant mutation, a second digestion was employed to eliminate the dominant mutant molecules. For juice samples containing dominant GAT mutation, the BstN1 digested product (1 µl) was then re-amplified for 35 cycles using the same forward primer, which bears a Bcc I restriction site in the present of mutant GAT *KRAS2*, and a reverse primer (5'-CCCTGACATACTCCCAAGGA-3' (SEQ ID NO: 22)) to produce a 304 bp PCR product. This amplified product was digested with 20 unit Bcc I (NEBL) at 37 °C for overnight. For samples with dominant GTT mutation, 1 µl BstN1 digested product was then re-amplified using a forward primer to produce an hpyCH4 III site for mutant GTT *KRAS2*, and digested (hpyCH4 III (NEBL), 37°C overnight). The BccI and hpyCH4 III digested products were further amplified and subsequently purified using Qiagen PCR purification kit. Hela DNA similarly treated as a negative control.

Please delete the paragraph on page 98, lines 11-21 and replace it with the following paragraph:

For DNA sequencing, HIV-1 RT domains in TyHRT elements were obtained by growing isolates overnight in 10 ml SC-URA at 30°C and preparing DNA with a glass bead/phenol extraction method. DNA was suspended in 50 μ l water and 0.5-1.0 μ l was used in a 50 μ l PCR reaction to amplify the RT domain. Amplification was carried out for 35 cycles (94°C for 1 min, 60°C for 1 min, 72°C for 2 min) using primers A-35 (5'GAACCTCCGAGATCGAAGA3' (SEQ ID NO: 23)) and I1097 (5'GCACTGCCTCTGTTAATTGT3' (SEQ ID NO: 24)) resulting in a PCR product that includes the HIV-1 RT coding region for amino acids 1-367. PCR products were purified with the Qiaquick 96 PCR Purification Kit (Qiagen Sciences, Germantown, MD) and sequenced with internal primers B275 (5'AGACTTCTGGGAAGTTCAAT3' (SEQ ID NO: 25)), 220F (5'TGGAGAAAATTAGTAGATTT3' (SEQ ID NO: 26)) and J801 (5'ATCCCTGGGTAAATCTGACT3' (SEQ ID NO: 27)).

Please delete the Table 5 header on page 99, line 1 and replace it with the following header:

Table 5. Oligonucleotides and primers used in the LigAmp assay* (SEQ ID NOS 28-31 and 9-11, respectively, in order of appearance)

Please delete the Table 7 header on page 108, line 6 and replace it with the following header:

Table 7. Oligonucleotides and probes (SEQ ID NOS 3, 6-7, 15, 17 and 9-12, respectively, in order of appearance)